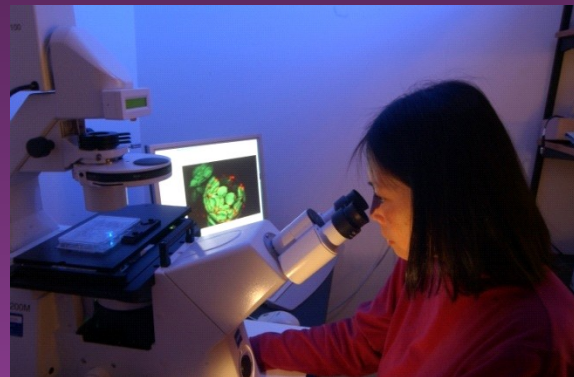
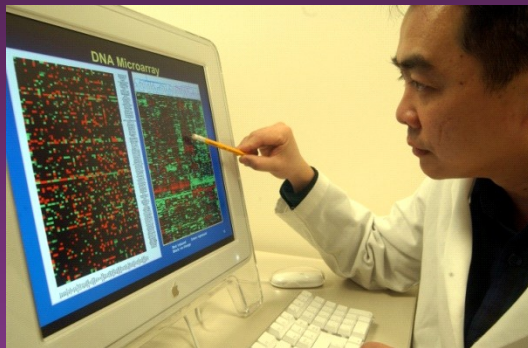




Driving the Future of Nutraceuticals

The Institute for Nutraceutical Research at Clemson University

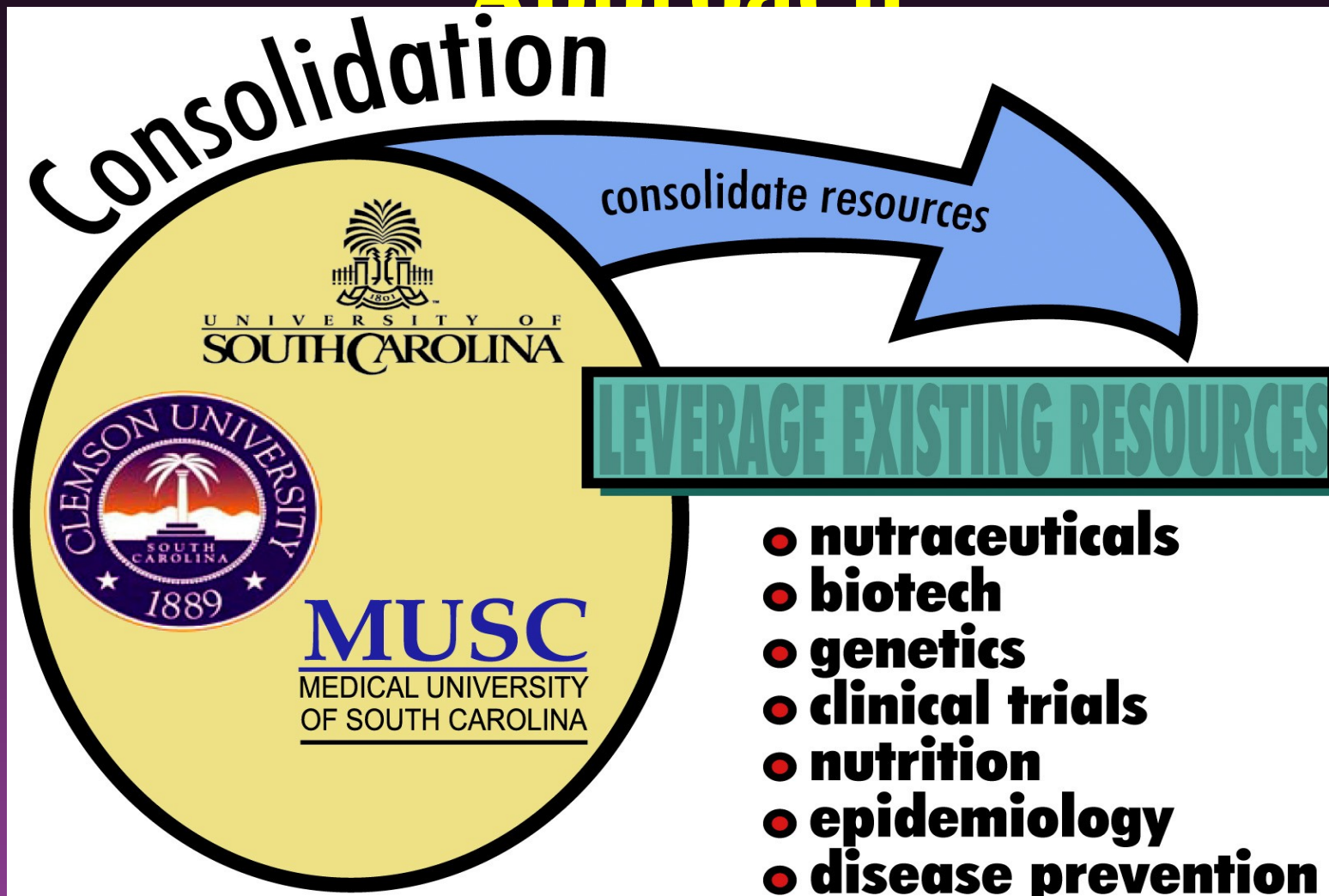




Driving the Future of Nutraceuticals

A Statewide Systems Biology Approach

Consolidation



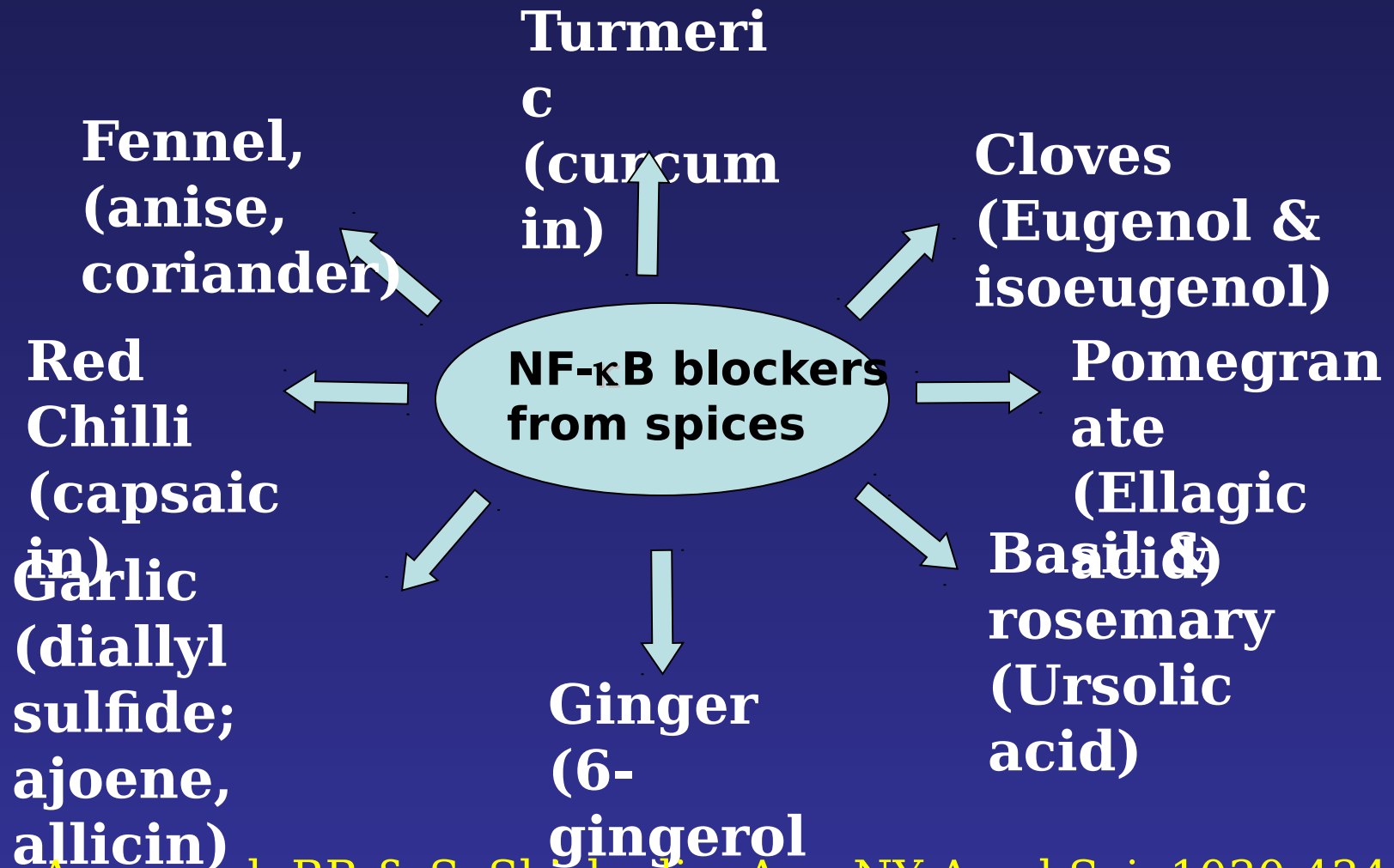
Food as Medicine



Medicinal Plants with Anti-inflammatory and Anti-oxidant Properties

Culinary Herbs	Vegetables	Traditional Medicinal Plants
Turmeric (curcumin) Cilantro/Coriander	Leafy & other crucifers	Muscadine
Yarrow	(broccoli, collards, kale etc.)	Neem
Fennel	Watermelon	Noni
Primrose	Tomato	Bitter gourd
Tarragon	Peppers	Mahogany
Garlic		Black cohosh

Suppression of NF- κ B and Inflammation by spice polyphenols, including the Indian spice turmeric (currie)



Aggarwal, BB & S. Shishodia. Ann NY Acad Sci. 1030:434-441, 2004

...to deep space



from deep sea...

*...to continue to be the preeminent provider of Joint Service,
Coalition, capability driven world-class combat feeding systems...*

by

“Cellular and Animal Model Screening of Potential Performance-Enhancing Phytonutrients”

DoD Combat Ration Research & Development

**“Basis for & extent which food constituents
and dietary supplements:**

- Delay fatigue
- Extend physical strength & endurance or
- Heighten alertness or enhance cognitive abilities of soldiers engaged in physically or mentally demanding tasks”



Driving the Future of Nutraceuticals

High Throughput Screening of Phytonutrients

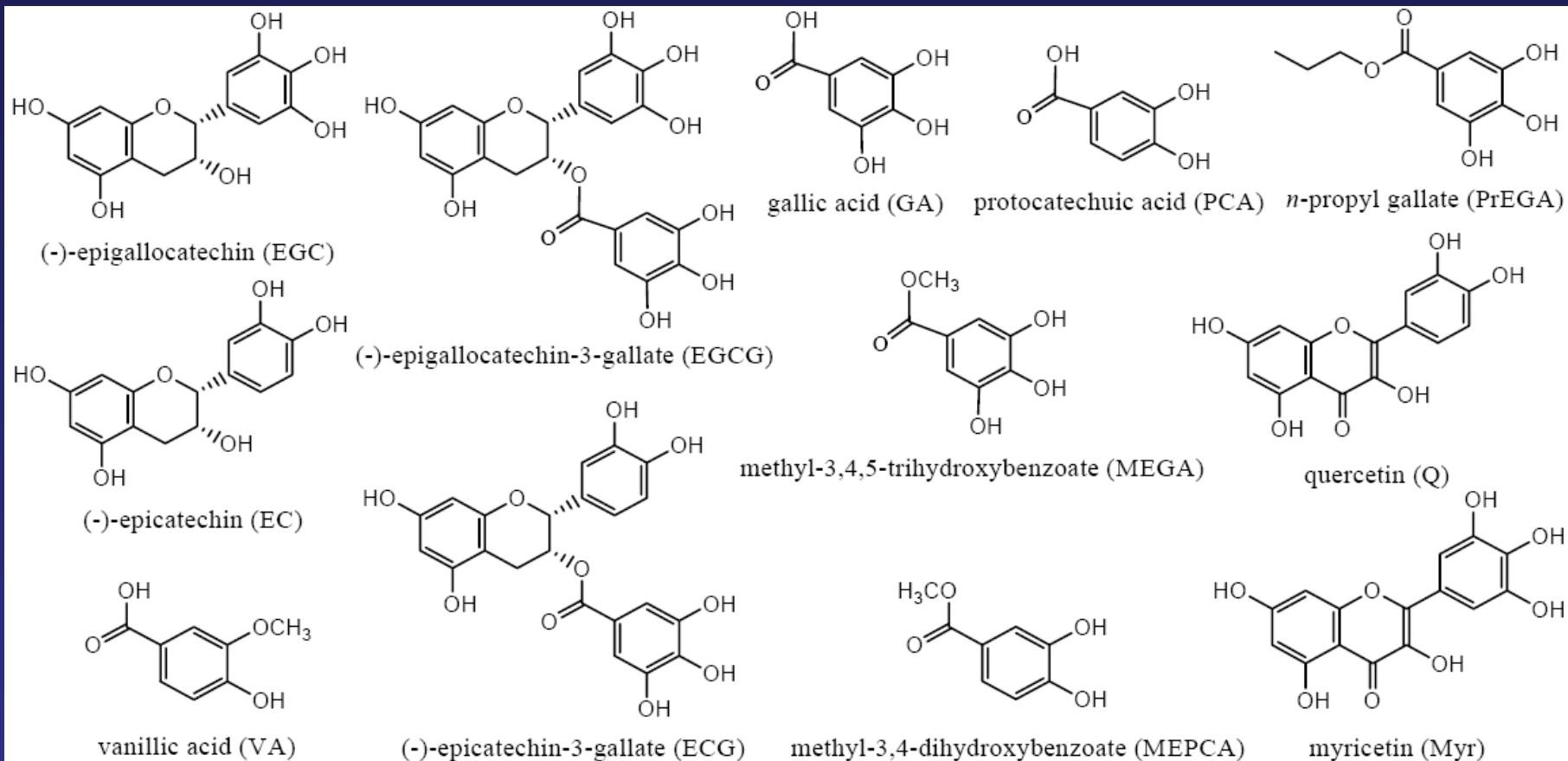
Level I- Immune Cell Markers

- **Pro-inflammatory and Anti-inflammatory cytokine release**
- **Antioxidant properties**
- **NF kappa B inhibition**
- **Prostaglandin inhibition**

Level II- Animal Models

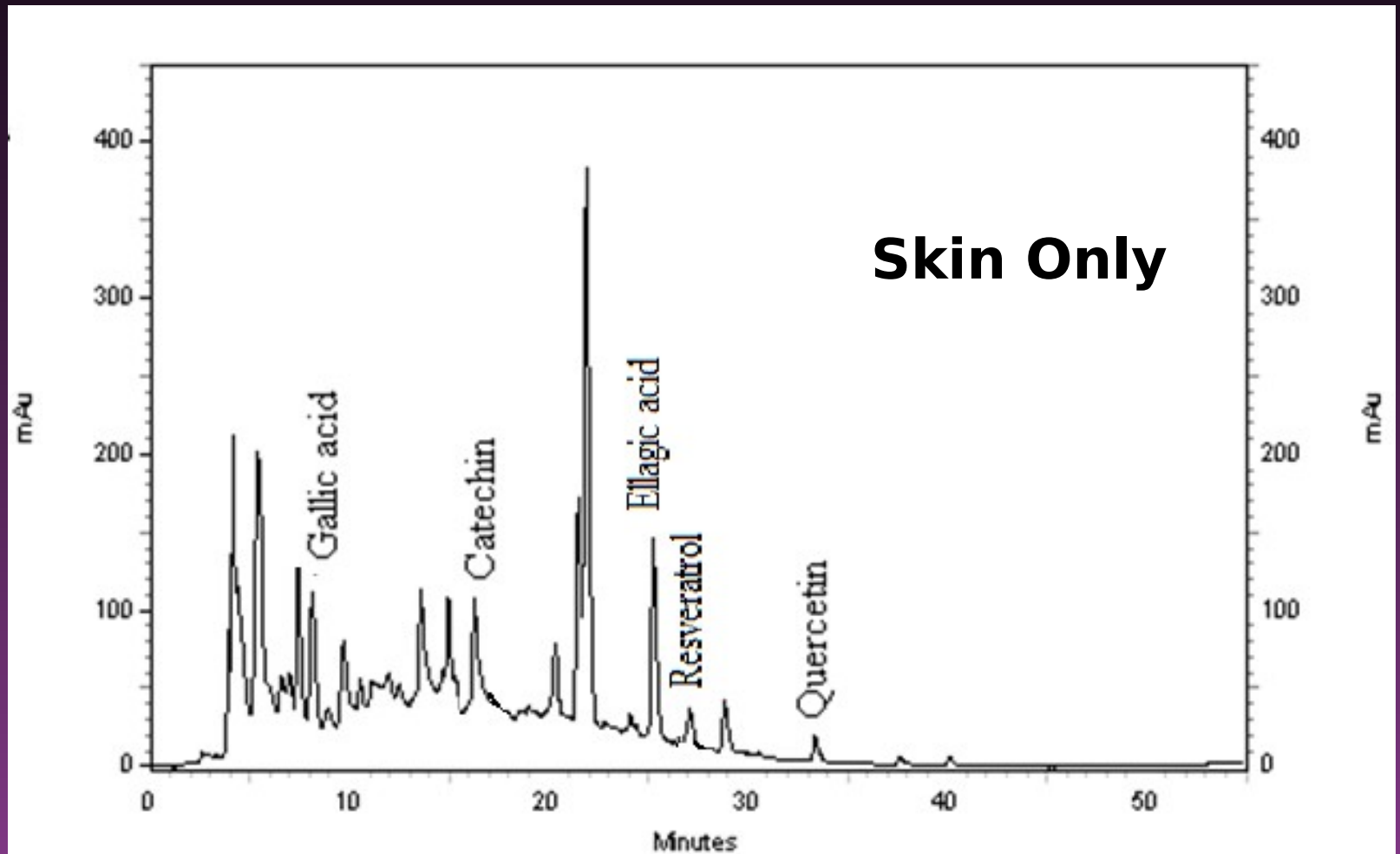
- **Physical stamina and recovery from fatigue**
- **Mental alertness and cognitive functions**
- **Resistance to viral infection**

Examples of Purified Polyphenols Tested



Driving the Future of Nutraceuticals

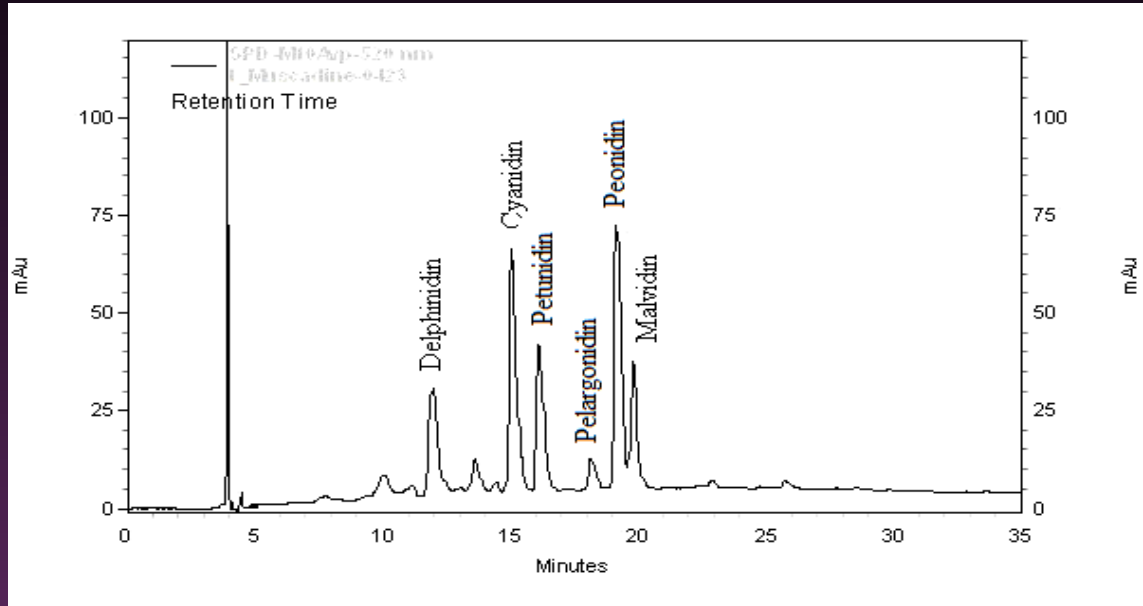
Example of Mixed Polyphenols in Muscadine



Muscadine Polyphenols (mg/100g FW)

	Seeds	Skins	Leaves	Pulp
<u>Antioxidant Capacity</u>	281**	13	236	3
<u>Total Phenolics</u>	2180**	375	352	24
Gallic acid	7		9	
Catechin	560*			
Epicatechin	1300*			
Ellagic acid		17*	67	
Myricetin		8	158*	
Quercetin		2	10	
Kaempferol		0.6	9	
Trans-resveratrol		0.1		
Anthocyanins		132*		

Anthocyanidins

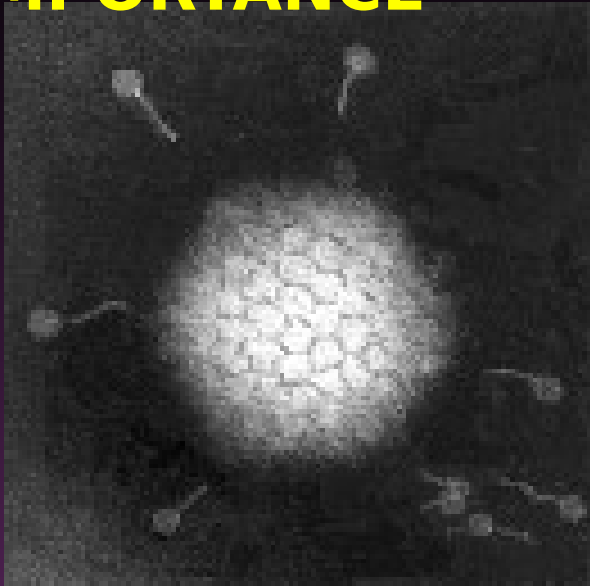


Compound s	mg/g of muscadine extract
Malvidin	10.39
Delphinidin	5.57
Cyanidin	3.62

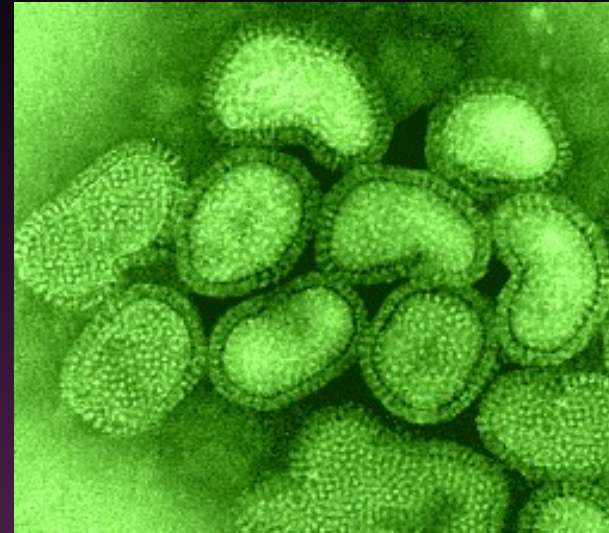
Promising Anti-Inflammatory and Anti-oxidative Nutraceuticals

<u>Agent</u>	<u>INF</u>	<u>áINF*</u>	<u>NF-kB</u>
<u>Recovery**</u>			
<i>Muscadine</i> +	-	++++	+++
<i>Curcumin</i> ++	-	+++	+
<i>Quercetin</i> +	-	+++	++
<i>Caffeine</i>	++	-	
<i>Echinacea</i>	+++	-	

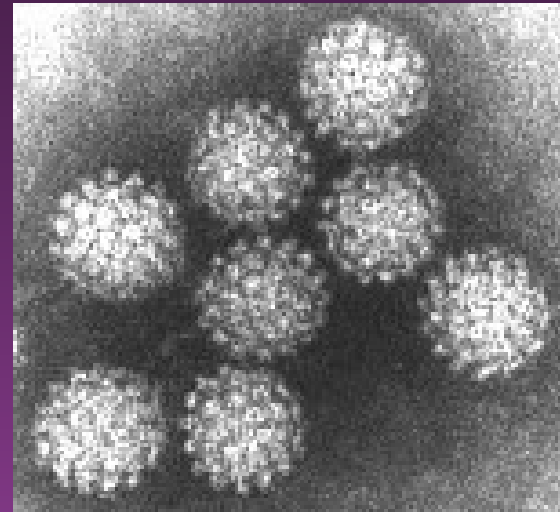
RESPIRATORY VIRUSES OF MILITARY IMPORTANCE



Adenovirus

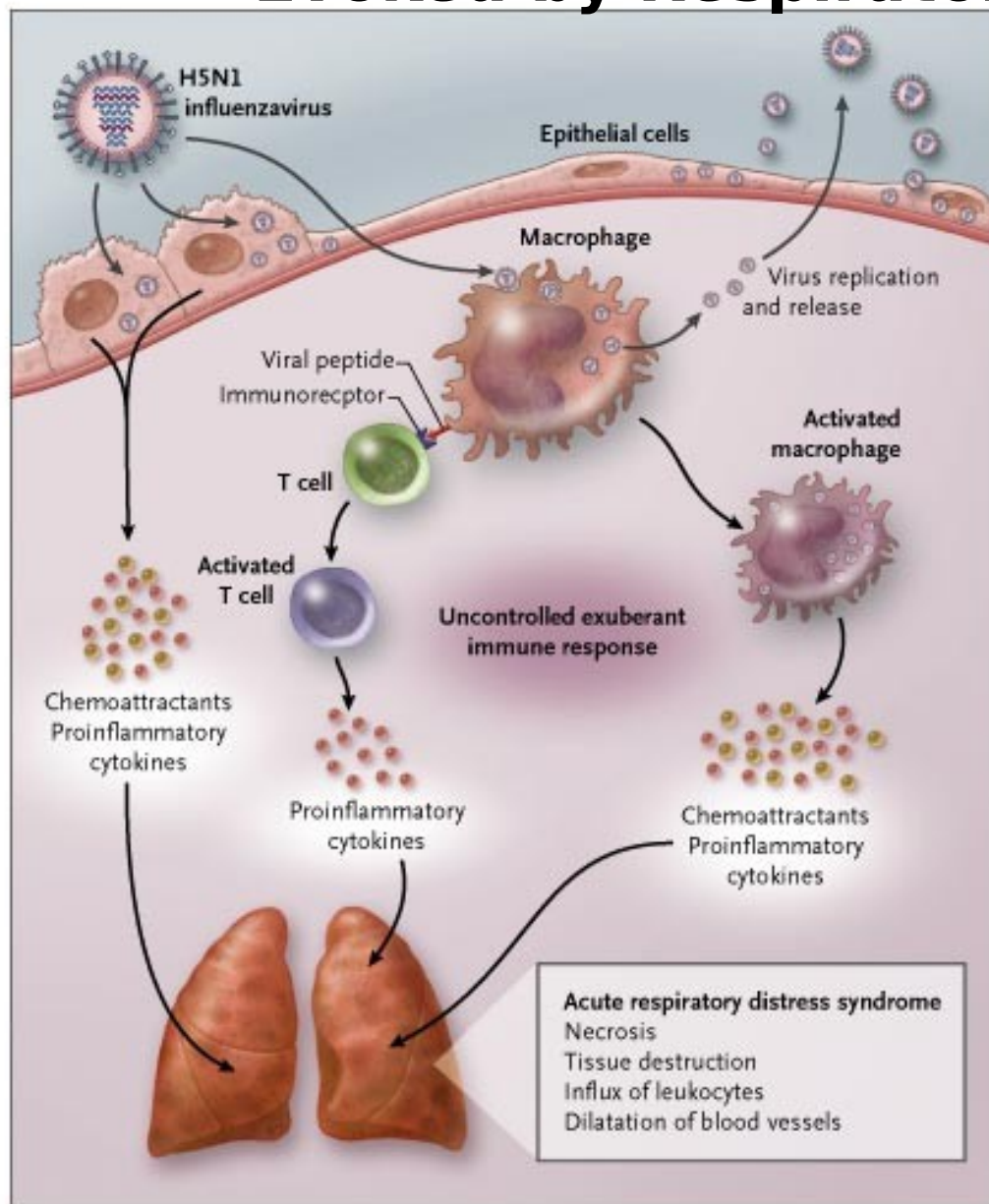


Influenza Virus



Rhinovirus

Proposed Mechanism of the Cytokine Storm Evoked by Respiratory Viruses

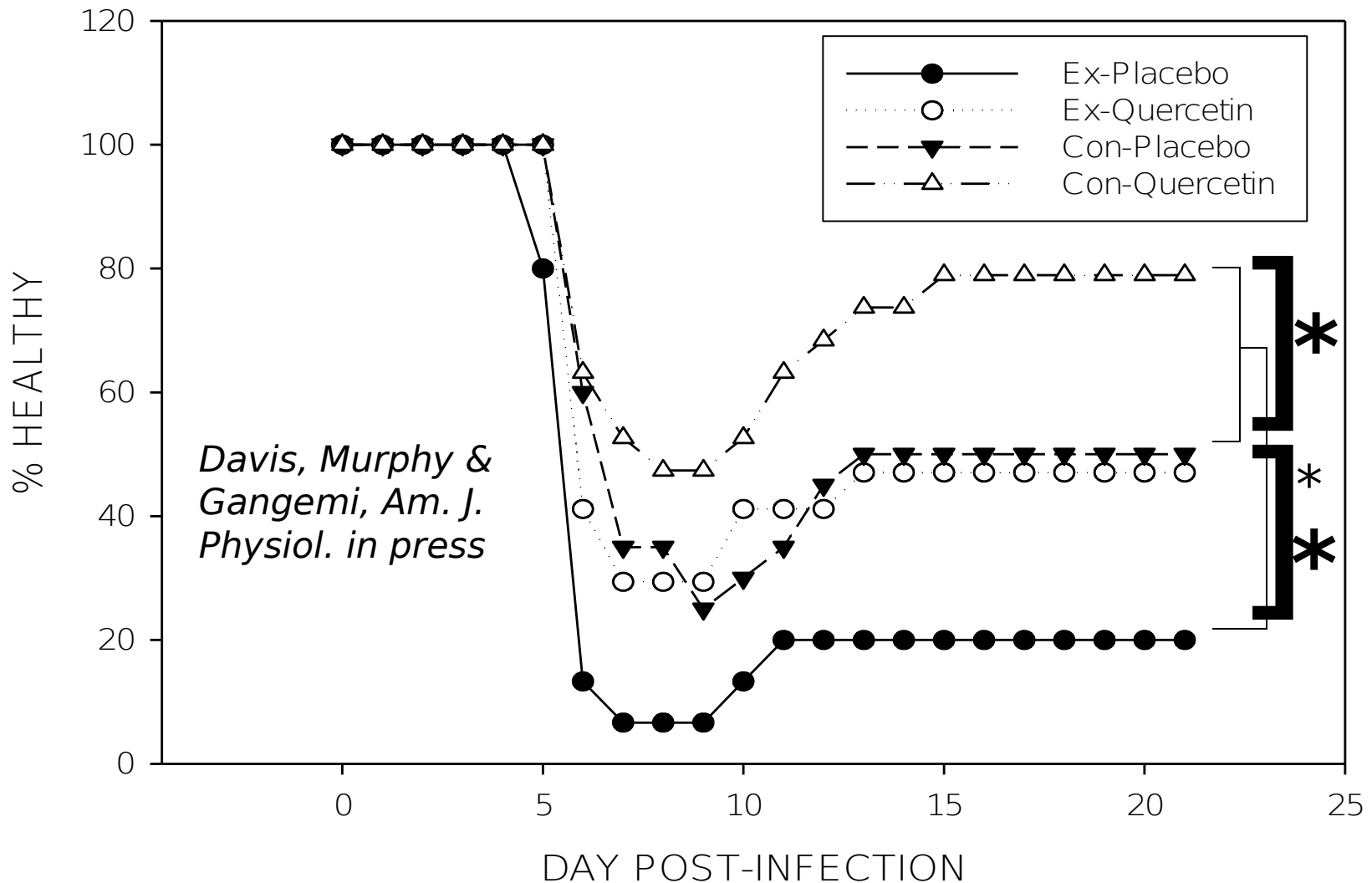


Osterholm, M. T.
N Engl J Med 2005;352:1839-1



The NEW ENGLAND
JOURNAL of MEDICINE

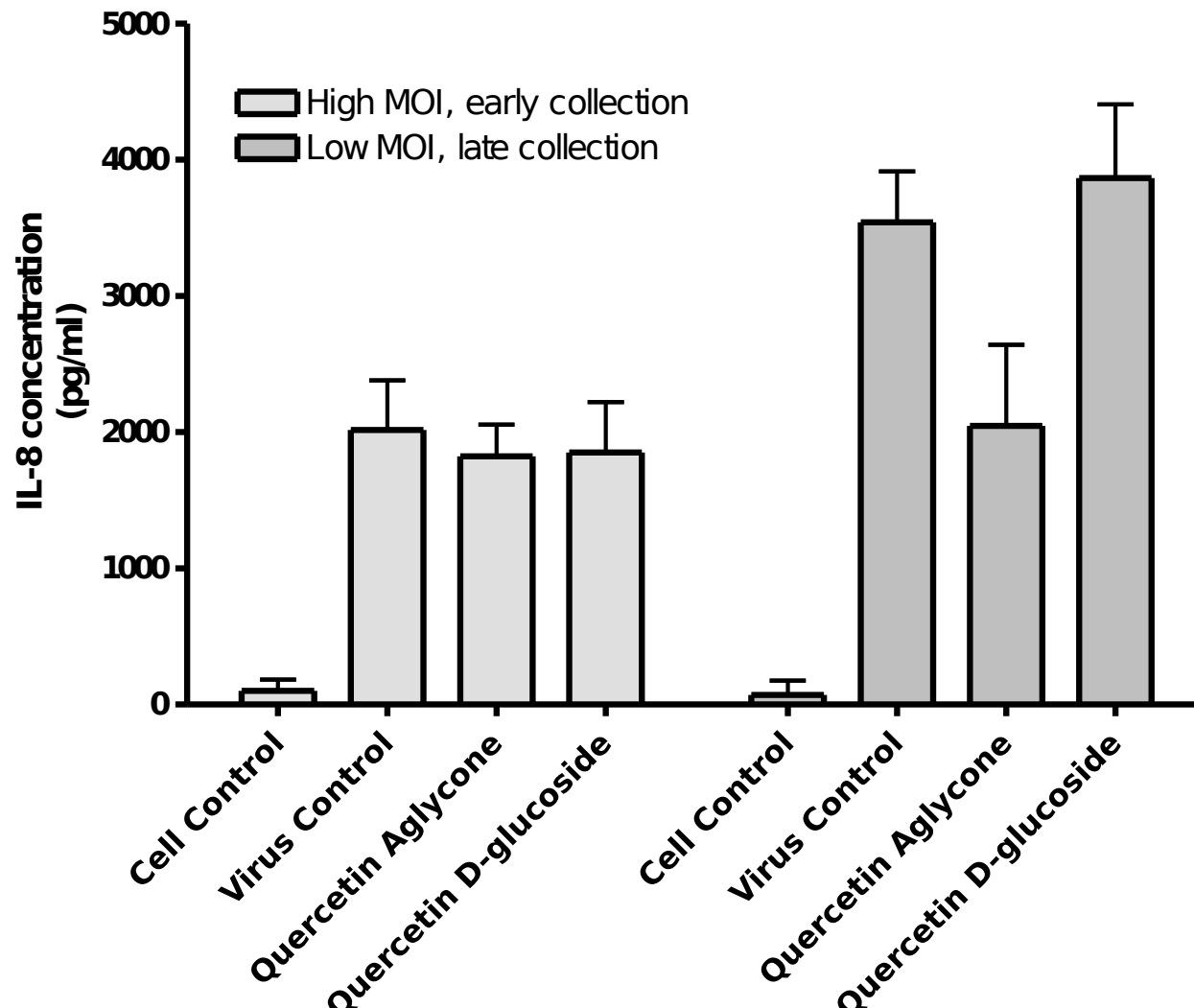
Quercetin Enhances Resistance to Influenza Following Exercise Stress



Key Elements in Rhinovirus Pathogenesis

- **Virus replication in nasal mucosal cells (temp. restricted, 33- 35° C)**
- **Replication induces oxidative stress in respiratory epithelium**
- **Activation of NF Kappa-B > amplifies immune response genes**
- **Induction of IL-8**
- **Recruitment of PMNs/degranulation**

Quercetin Suppression of Rhinovirus-Induced IL-8 Elaboration in Respiratory Cells



Next Steps

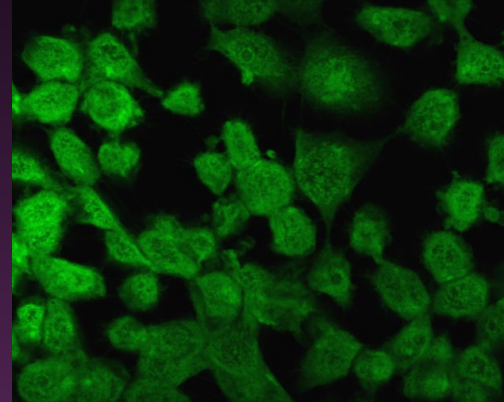
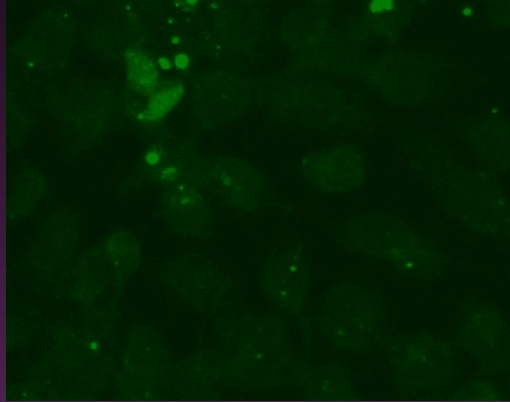
- Confirm antiviral activity in experimental adenovirus infections
- Examine clinical effects in natural cold model (adenovirus and rhinovirus)
- Evaluate alternative delivery models (i.e. buccal wafers) for improved bioavailability
- Genetically modify plants (e.g. muscadine) to produce higher concentrations of secondary metabolites

Brain/Muscle Food - Best Bets

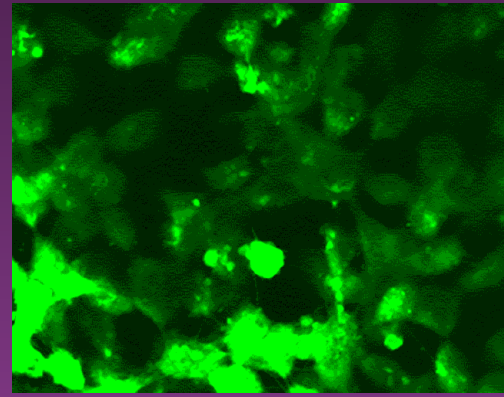
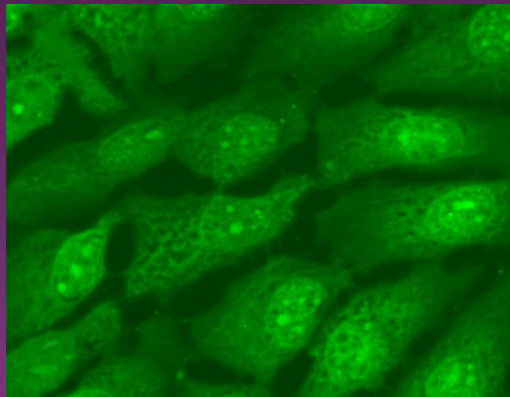
- **Carbohydrates** – Good bet to delay mental and physical fatigue
- **Caffeine** – Good bet to delay mental fatigue
 - Optimal doses vary widely and side effects can be serious in high doses, especially if combined with other stimulants
- **Quercetin** – Good evidence of a benefit on mental and physical fatigue
- **Curcumin** – Some evidence of faster performance recovery following muscle damage
- **Muscadine Extract** – Rising fast

Cellular Oxidant Stress in Response to Rhinovirus Challenge

Cell control



Rhinovirus
Challenge
d



Carbonyl stain

DCFDA stain



Quercetin Chews



Placebo Chews



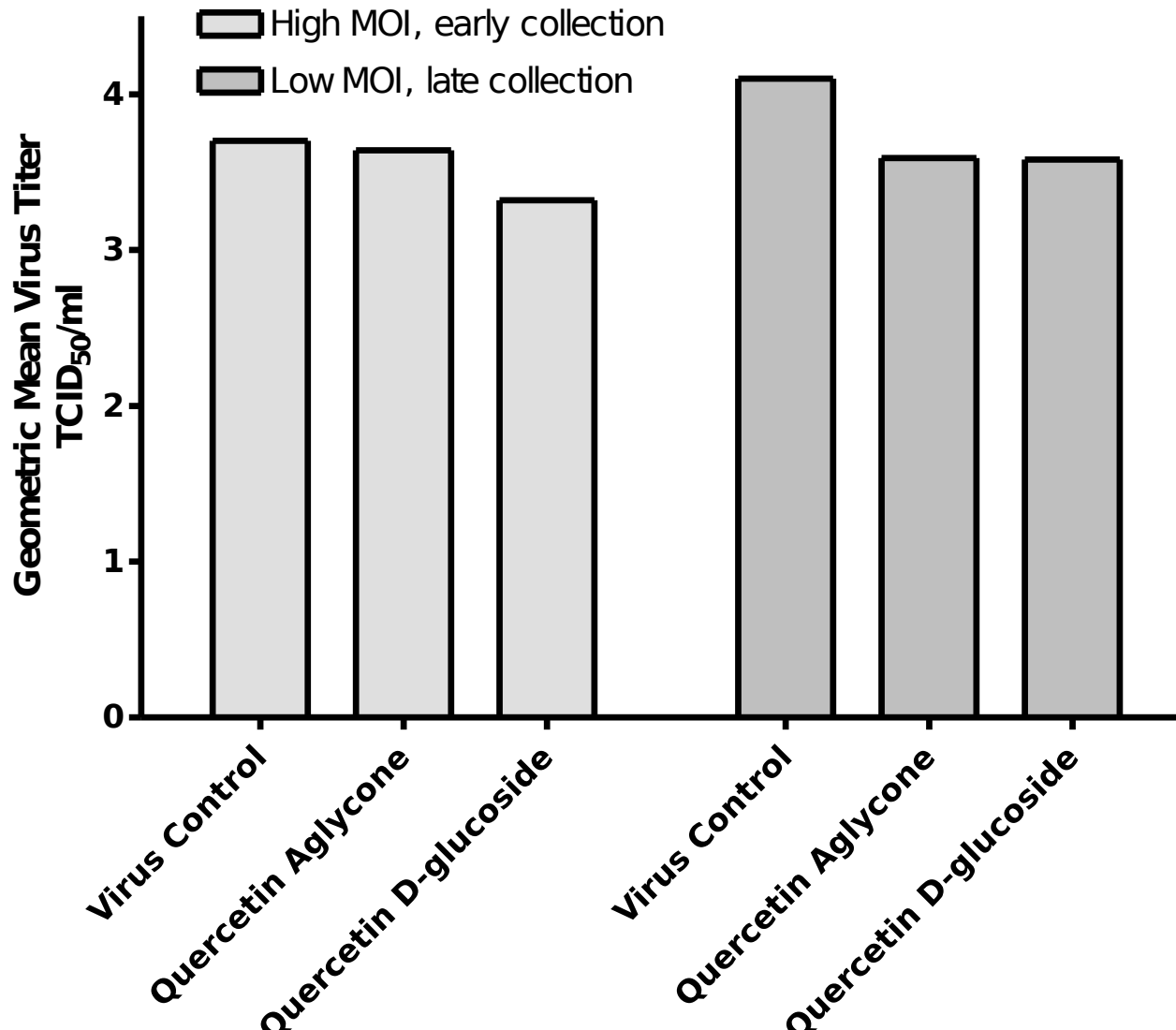
Curcumin Chews

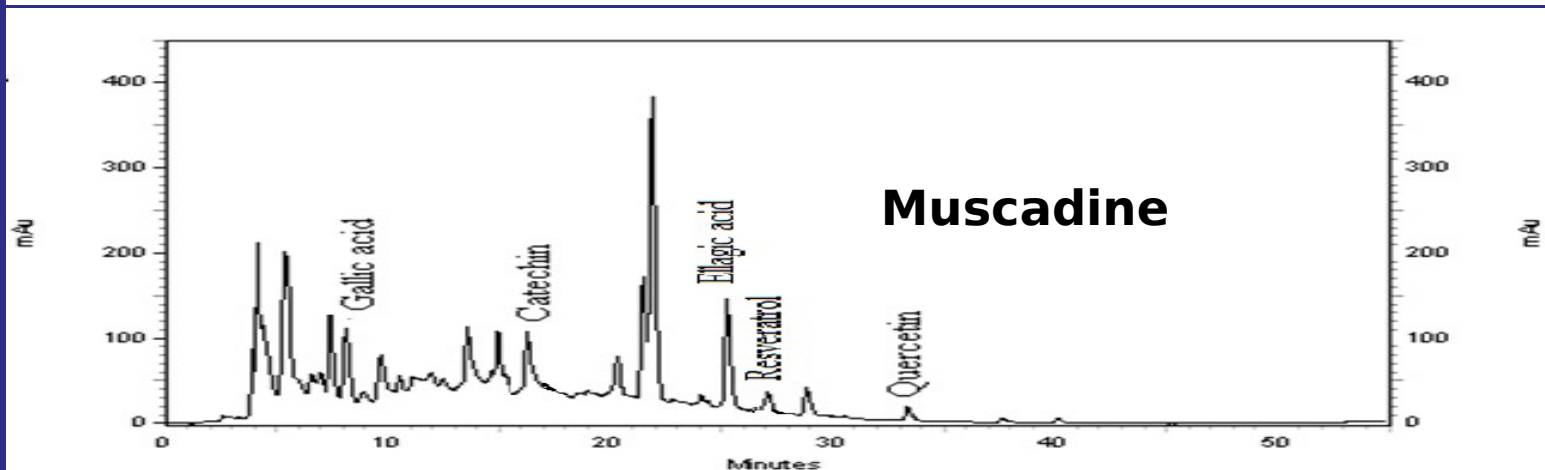
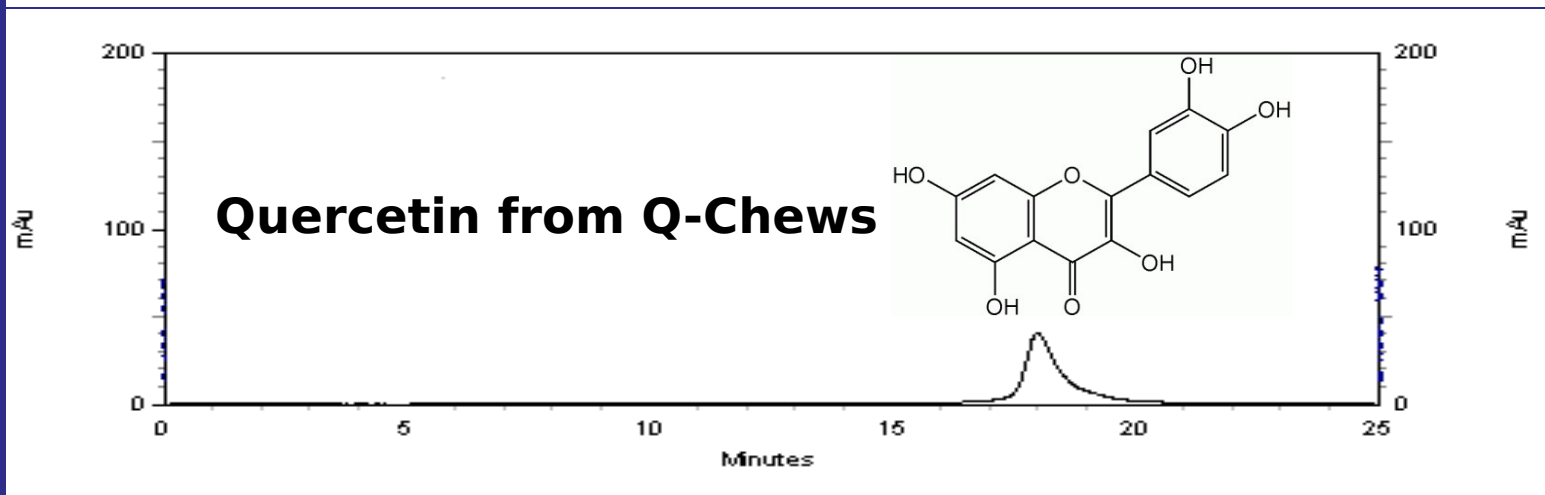
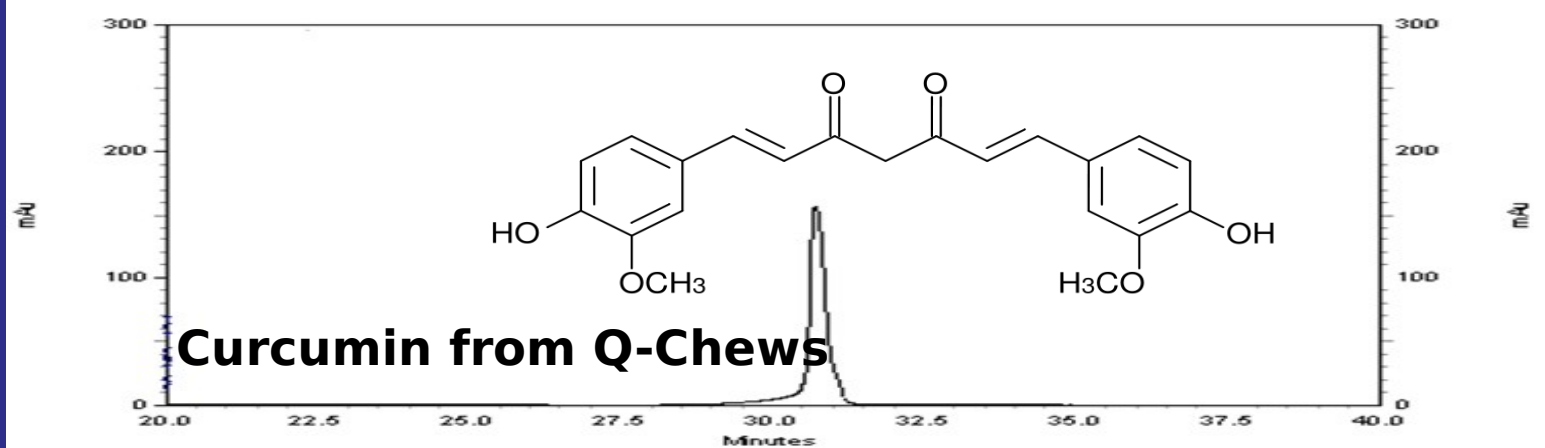


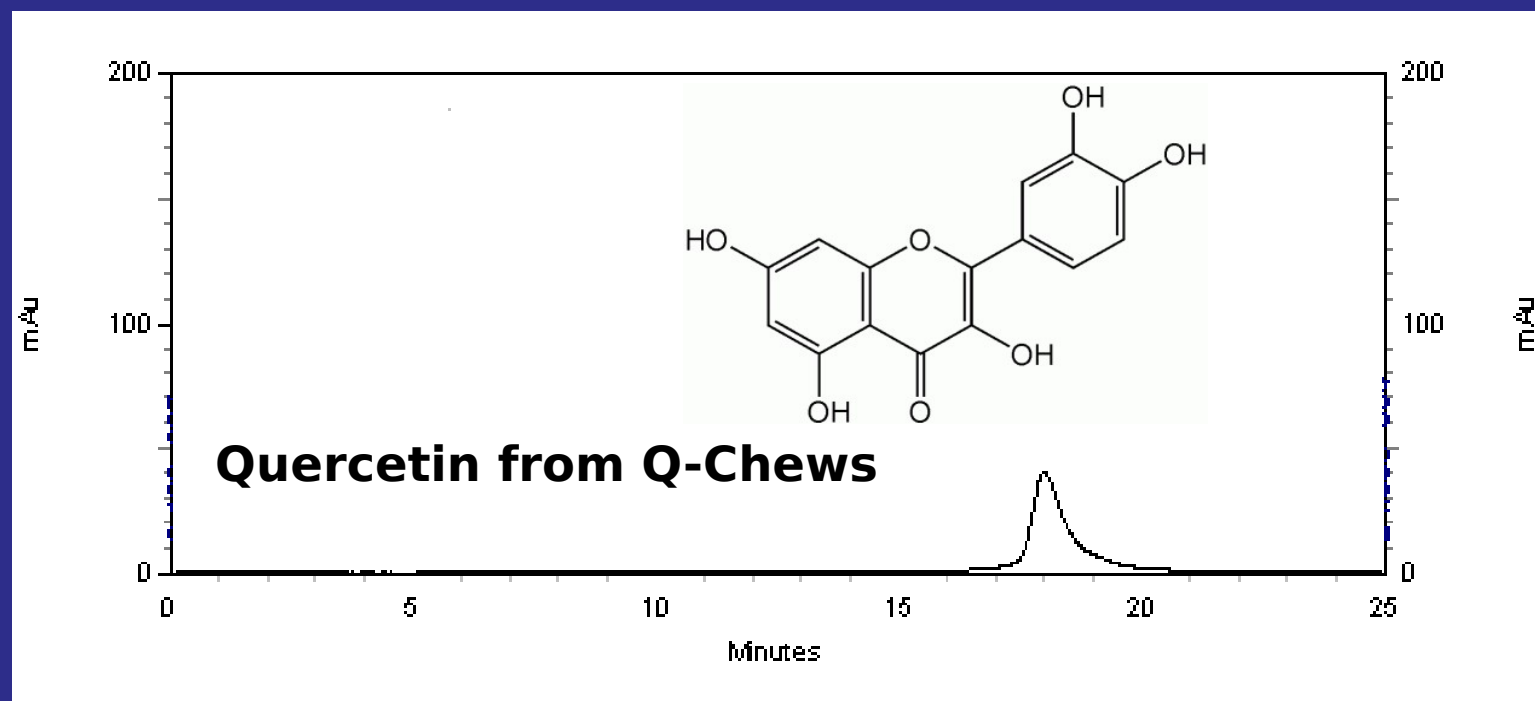
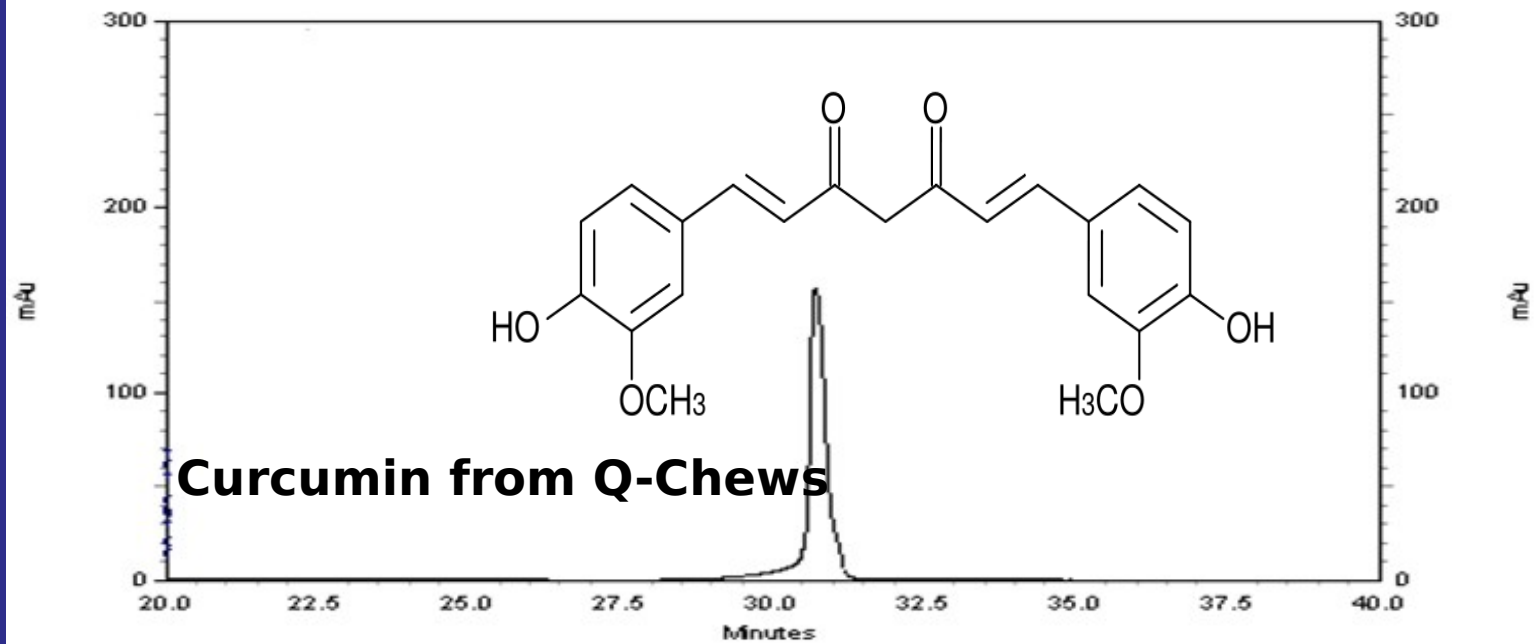
Quercetin Chews

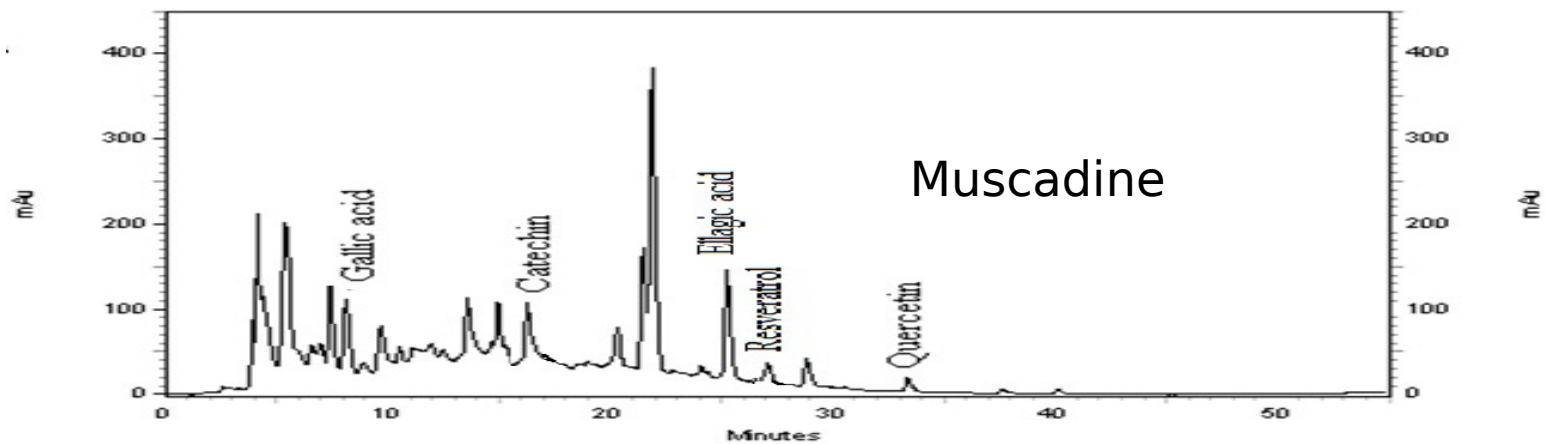
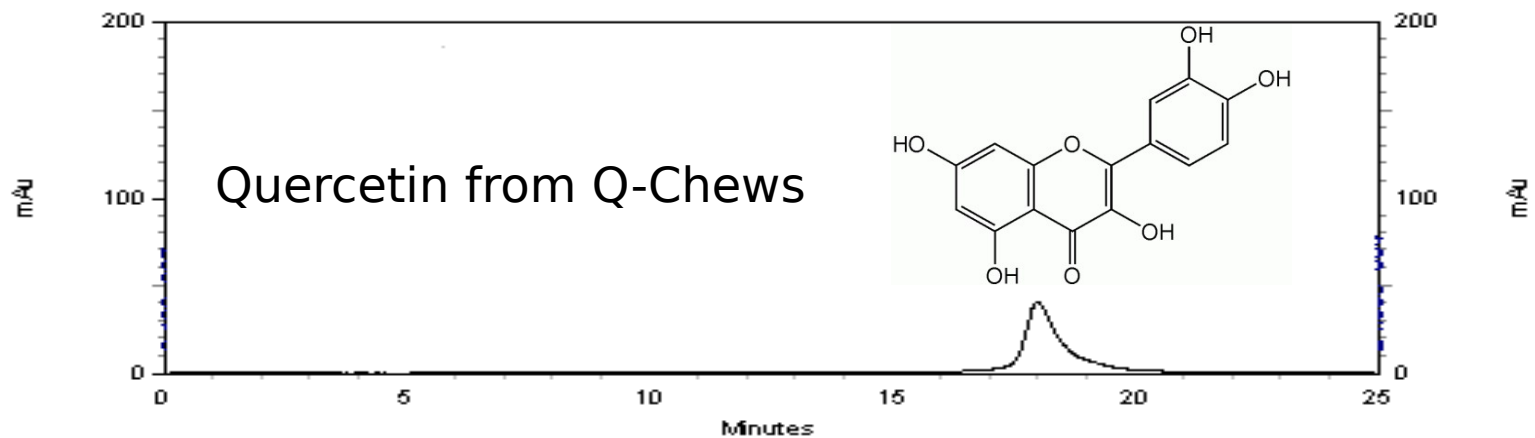
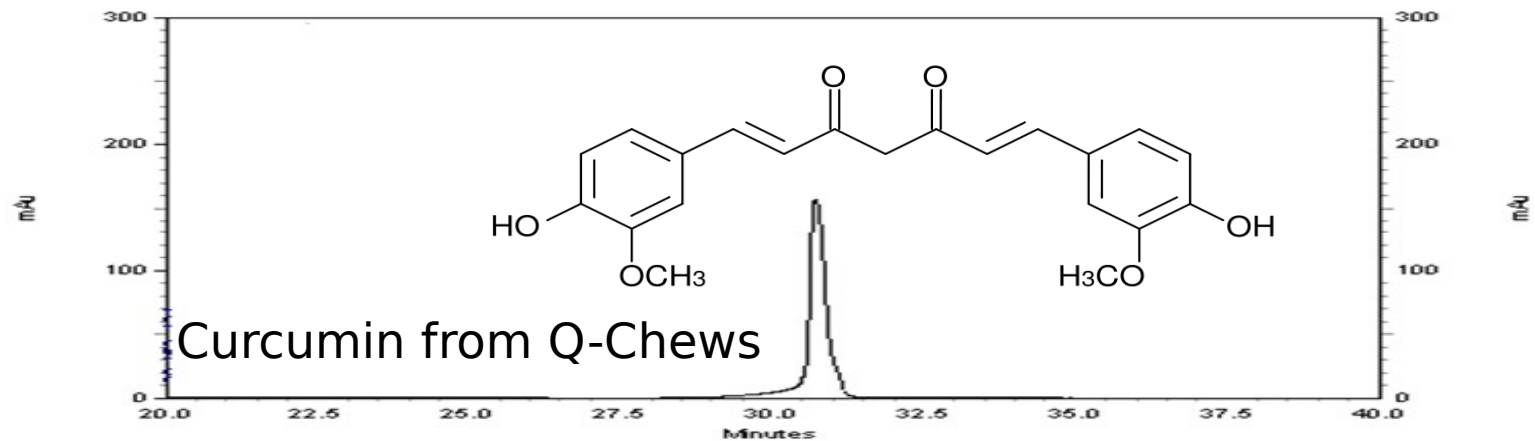


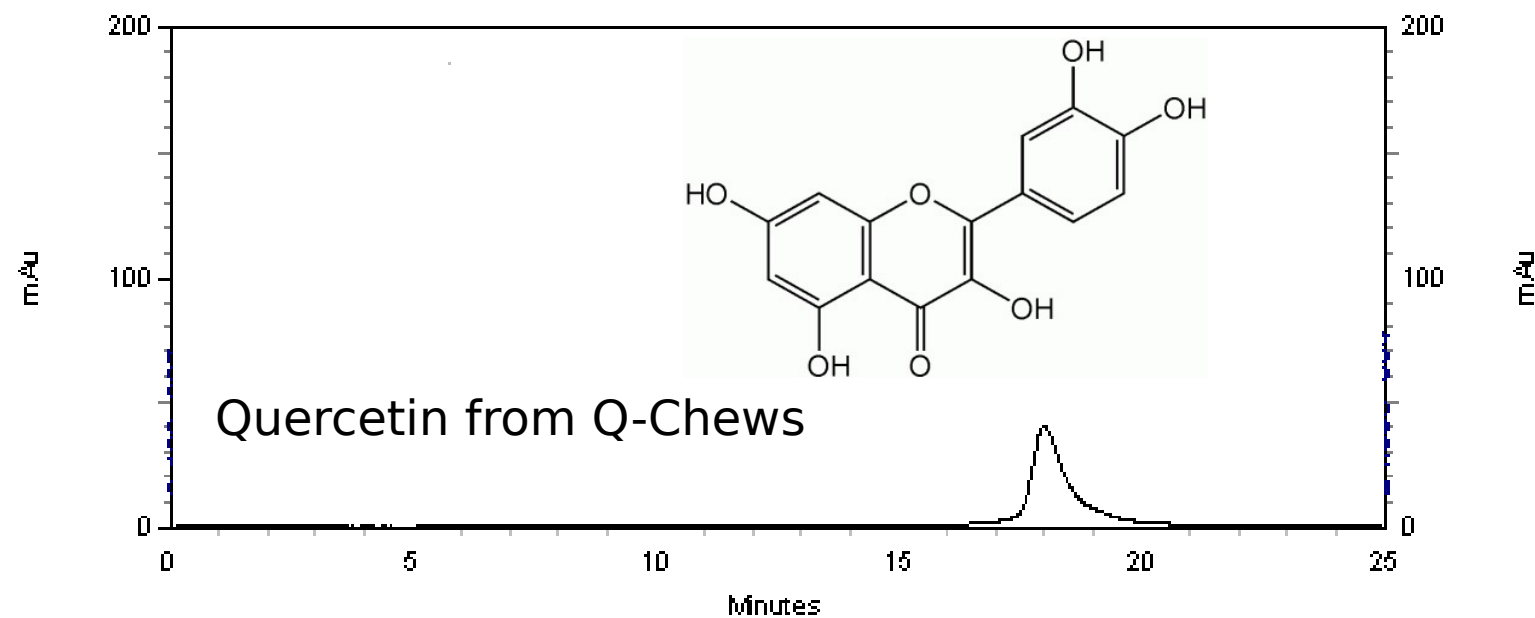
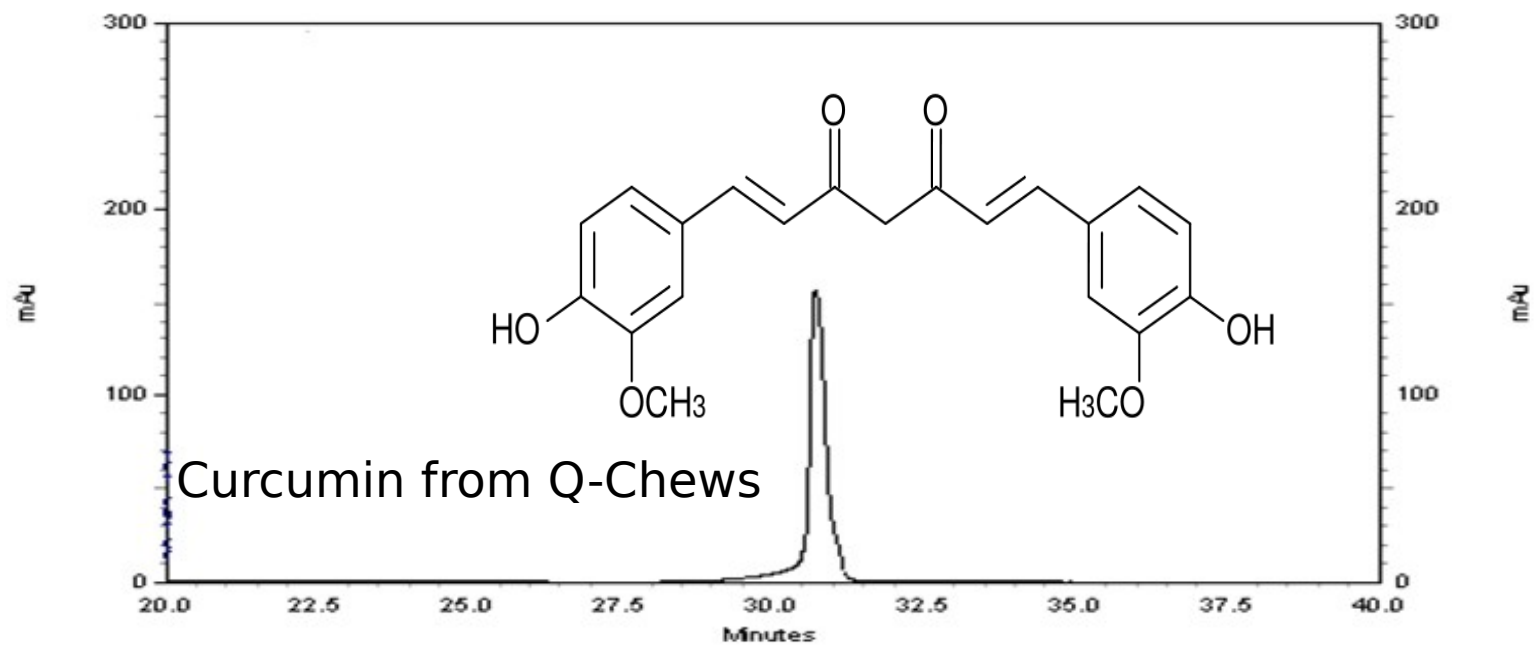
Effect of Quercetin on Rhinovirus Replication in Respiratory Cells



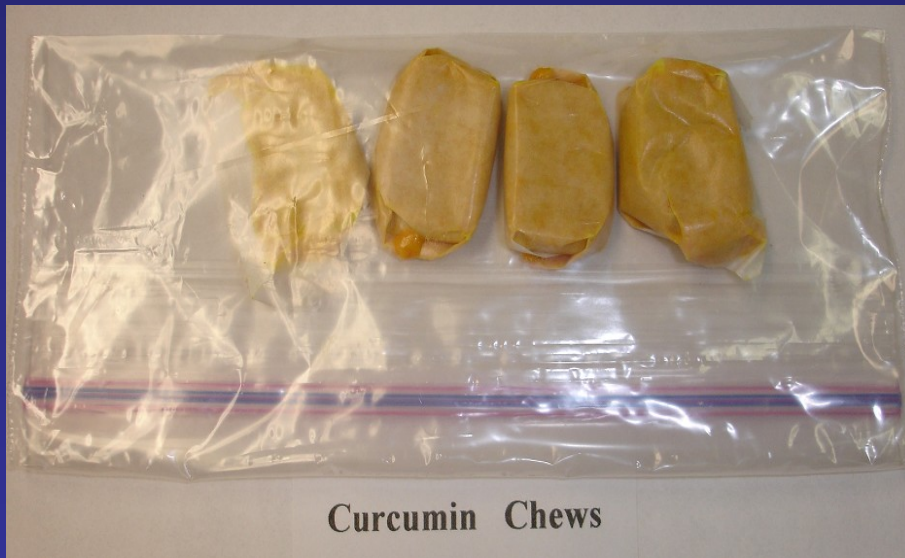




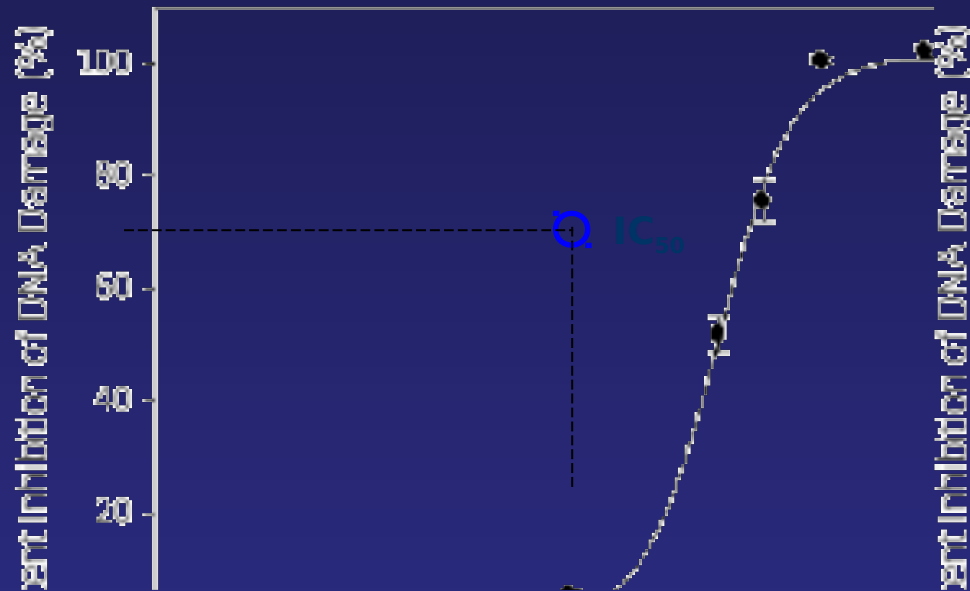




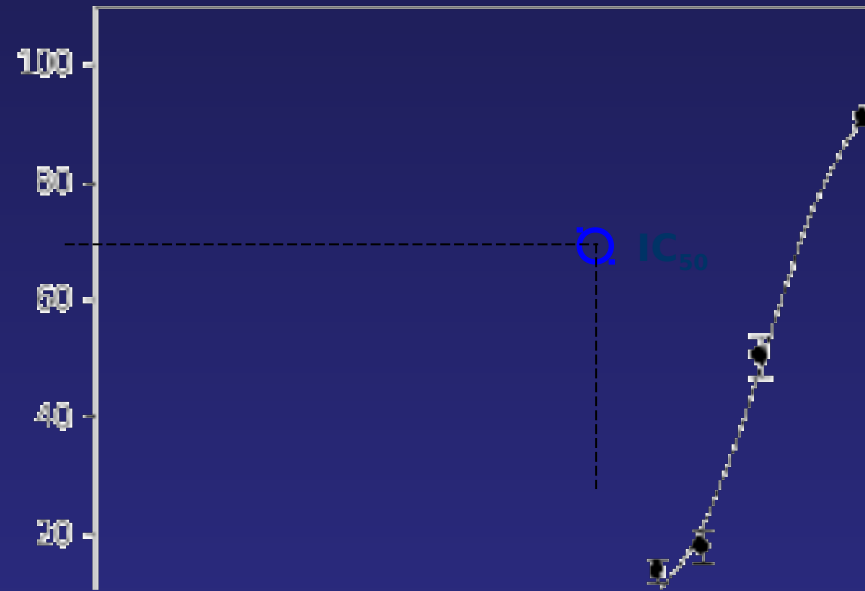
Q-Chews



Inhibition of DNA Damage by Myr and Q



$$IC_{50} = 2.0 \pm 1 \mu M$$



$$IC_{50} = 10.8 \pm 1 \mu M$$

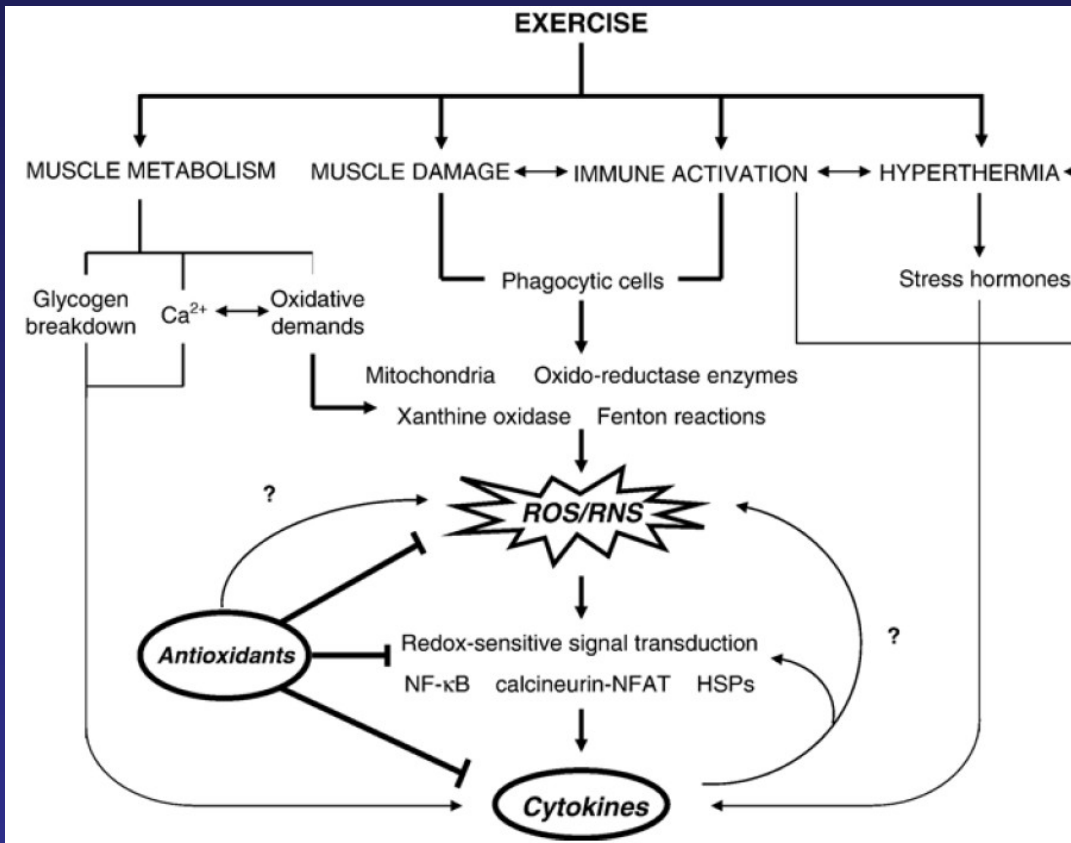
Physiological concentrations of polyphenols commonly reach
1-10 μM

Yamamoto, T.; et al. *J. Pharm. Exp. Ther.* **2003**, 307(1), 230-236. Scalbert, A. and Williamson, G. *J. Nutr.* **2000**, 130, 2073S-2085S.



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Fatigue and Oxidative Stress



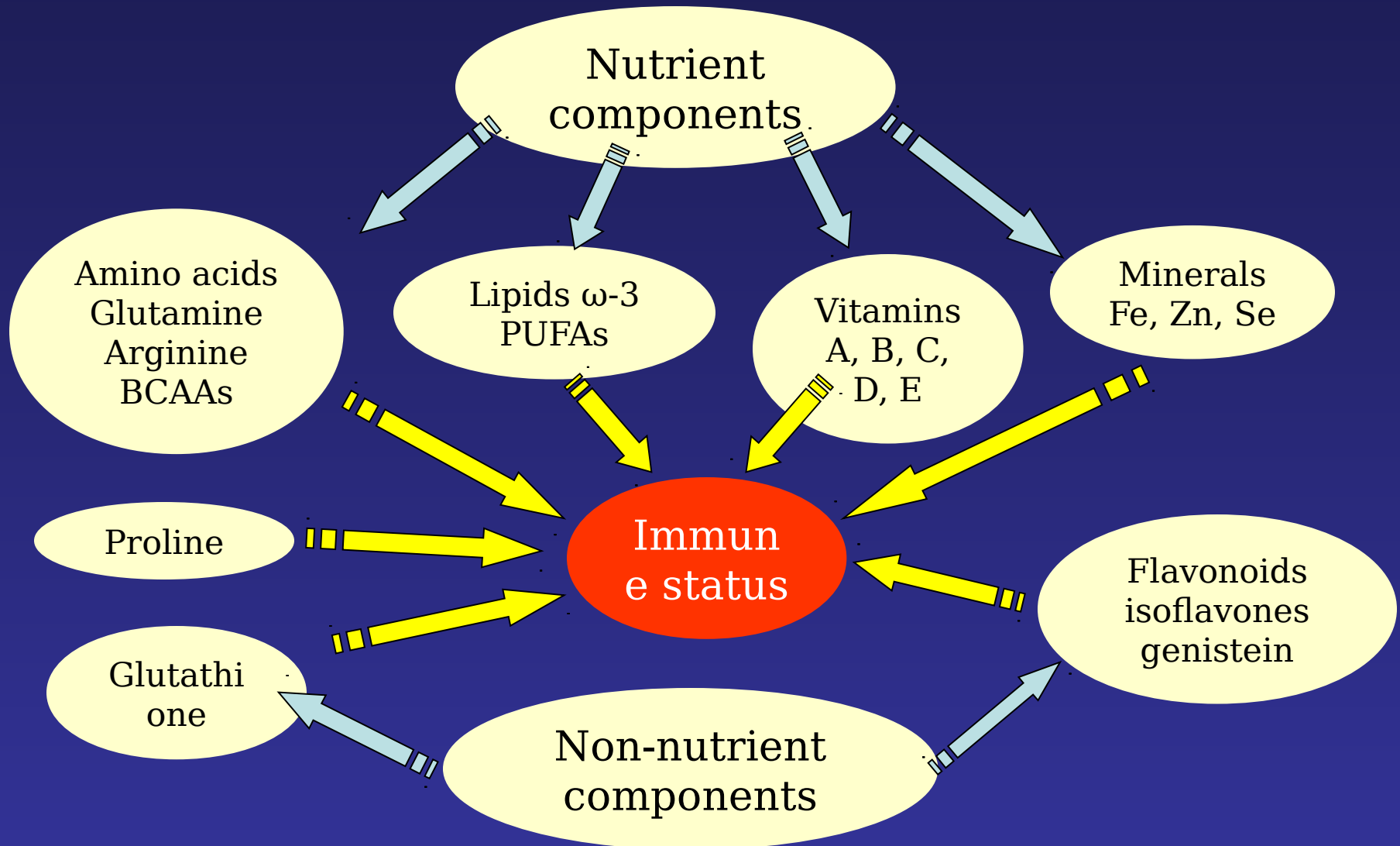
- Quercetin improves cycling time trial performance in humans
- Green tea supplementation reduced muscle lipid peroxidation in aerobically exercised rats

Peake, J.M.; et al. *J. Nutr. Biochem.* **2007**, 18, 357-371; Urso, M.L.; et al. *Toxicology* **2003**, 189, 41-54; Leeuwenburgh, C.; et al. *Curr. Med. Chem.* **2001**, 8, 829-838. MacRae, H. S.-H.; et al. *Int. J. Sport Nutr. Exer. Metab.* **2006**, 16(4), 405-419. Chai, Y.-M.; et al. *J. Food Sci. Nutr.* **2003**, 8(4), 377-382.



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Food factors affecting immune status



Conclusions

- 100% DNA damage inhibition is observed for all polyphenols at concentrations of 50 – 500 μM with $\text{Fe}^{2+}/\text{H}_2\text{O}_2$
- Gallate compounds were more potent than their catecholate analogs in every instance
- IC_{50} values correlated to pK_a values of the most acidic phenolic hydrogen for catecholate compounds
- Both iron-binding and lipophilicity are important factors: iron binding determines antioxidant activity, while lipophilicity determines bioavailability
- Have also developed protocols for testing antioxidant activity against DNA damage from both $\text{Cu}^+/\text{H}_2\text{O}_2$ and peroxynitrite



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HOST DEFENCE SYSTEM

Innate

Surface epithelia
Alternative pathways of complement activation
Macrophages (monocytes)
Dendritic cells in skin and lymphoid tissue

Adaptive

lymphocytes

B

Plasma cells
antibody producing

T

activated subsets

CD4

CD8

cytokines

Non-adaptive

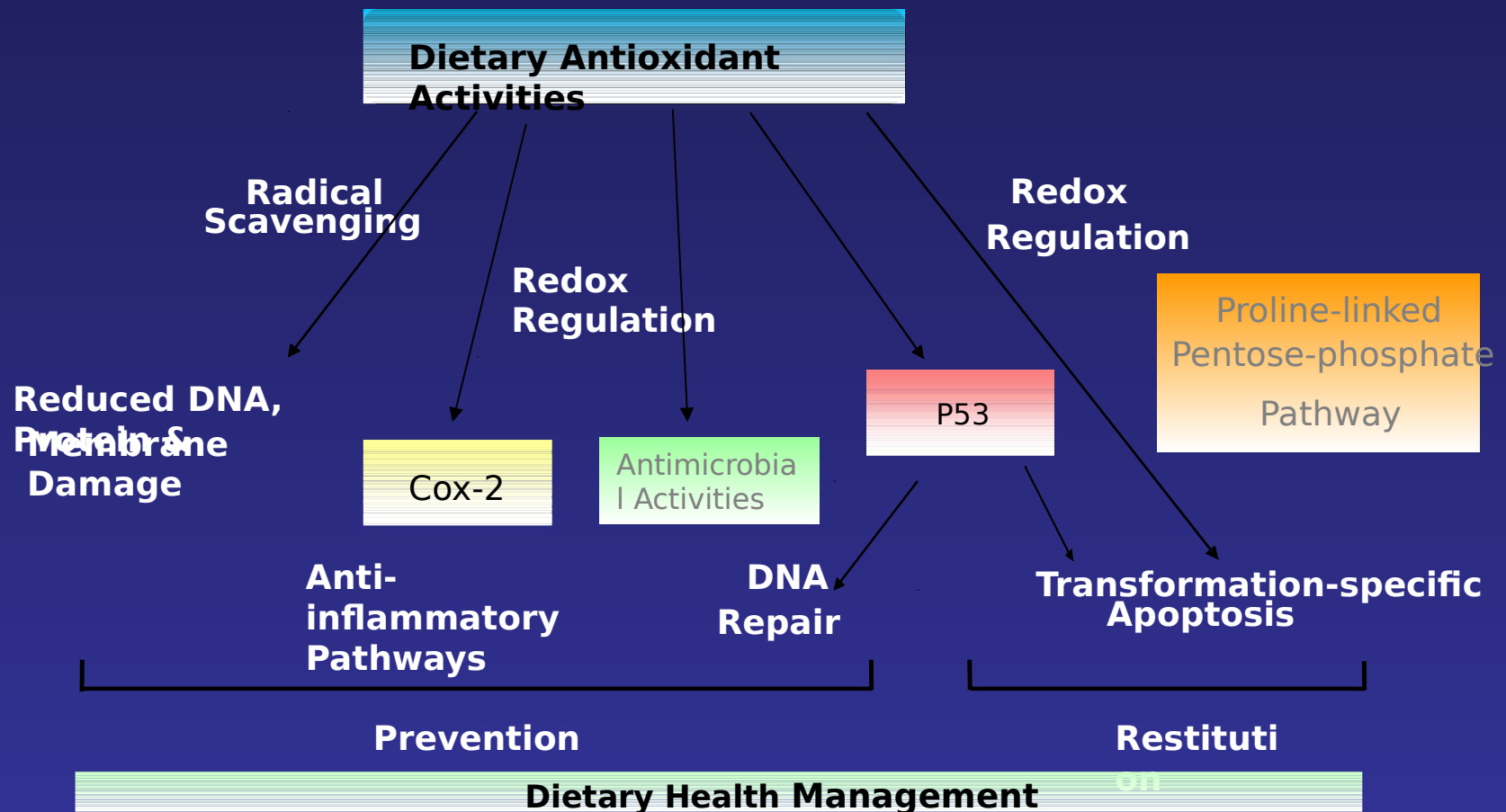
interferons

NK cells (natural killer cells) and certain B + T cells

Chemopreventive activities of antioxidants

(Food Ingredients to Stimulate Protective

pathways)
(Adapted from Brash and Havre, PNAS 2002; 99,13969)



**“It isn’t what
you eat that
can kill you,
and it isn’t
just your DNA
that can save
you- it’s how
they interact”
*Jose Ordovas, 2006***



Research Partnership For Preventing Cancer With Botanicals

MUSC
MEDICAL UNIVERSITY
OF SOUTH CAROLINA

Discovering... Understanding... Healing...

 **Coastal Research & Education Center**
CLEMSON UNIVERSITY PUBLIC SERVICE ACTIVITIES

CLEMSON
UNIVERSITY

**“SC Grown”
Fruits and Vegetables**



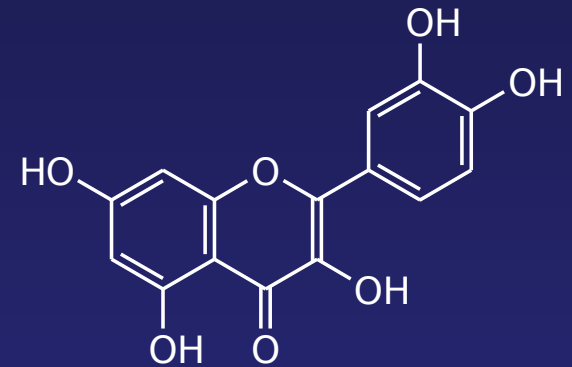
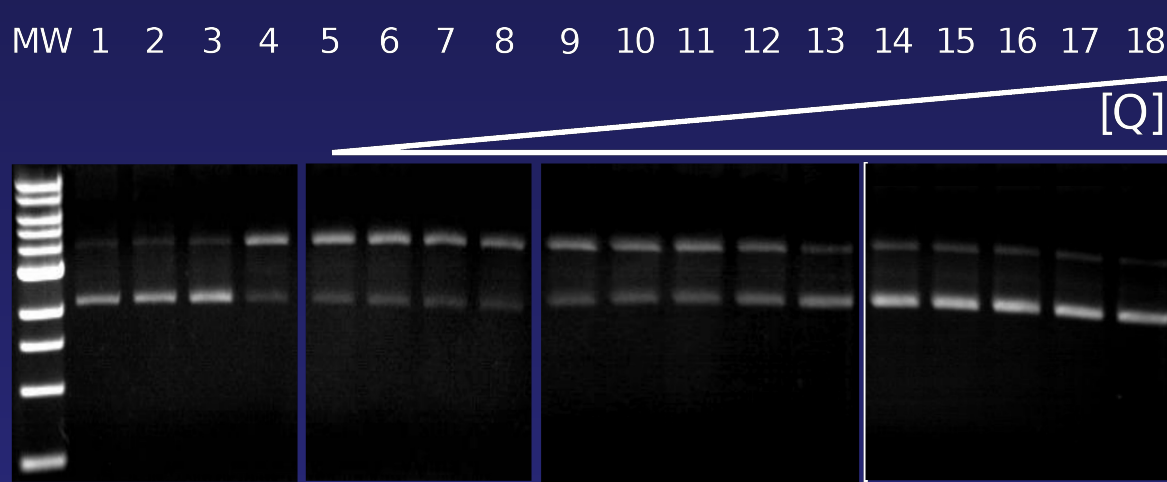
**“SC Grown”
Culinary Herbs**



**Traditional Medicinal
Plants**



Effect of Quercetin on DNA Damage



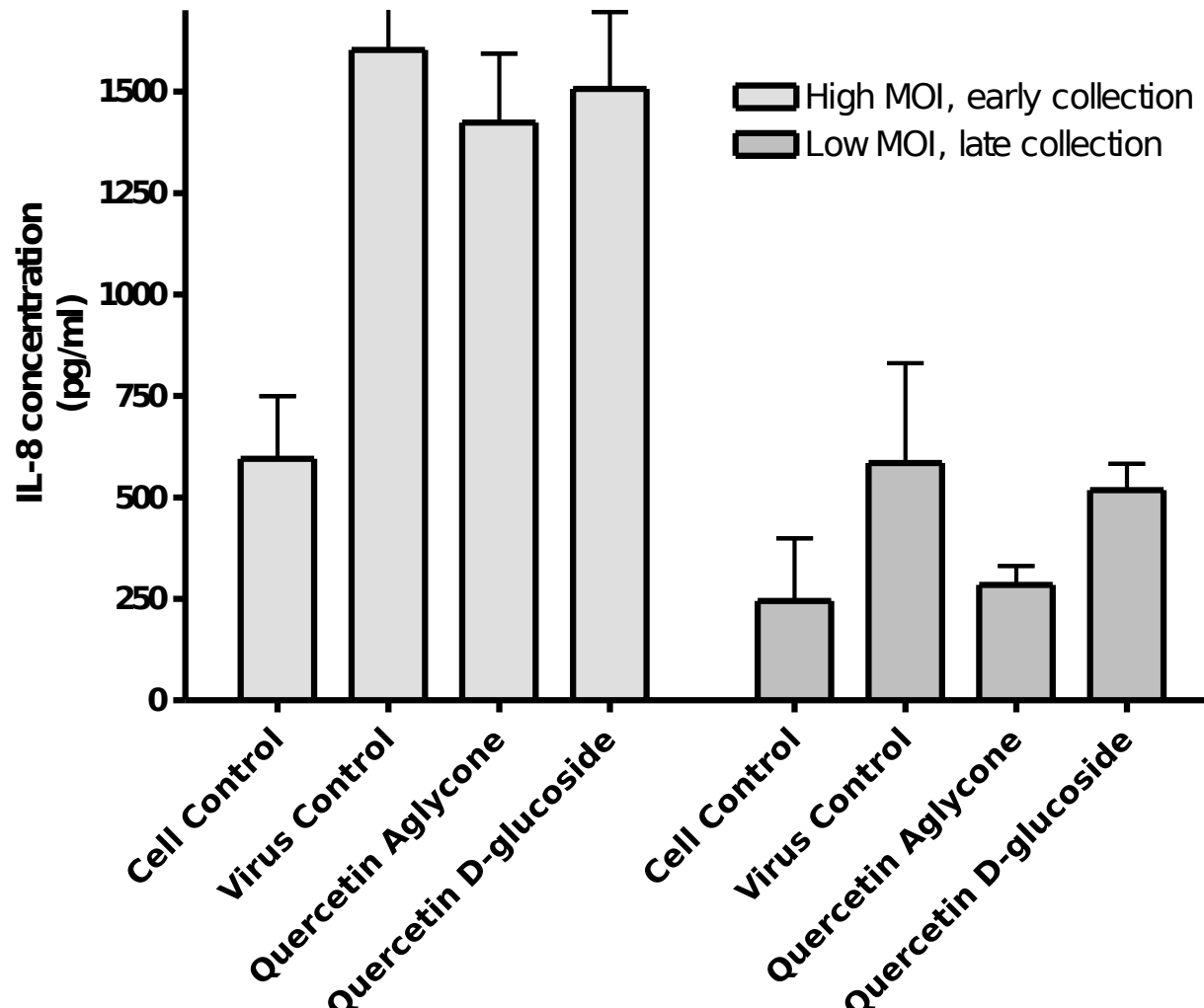
Quercetin (Q)

- Control Lanes: **MW** = 1 kb DNA ladder; **1** = plasmid DNA; **2** = DNA, 50 μM H_2O_2 ; **3** = DNA, 500 μM Q, 50 μM H_2O_2 ; **4** = DNA, 2 μM Fe^{2+} , 50 μM H_2O_2
- Experimental Lanes: **5-18** = DNA, 2 μM Fe^{2+} , 50 μM H_2O_2 , and increasing Q concentration (0.001, 0.002, 0.02, 0.05, 0.1, 0.2, 2, 4, 10, 50, 100, 200, and 500 μM , respectively)

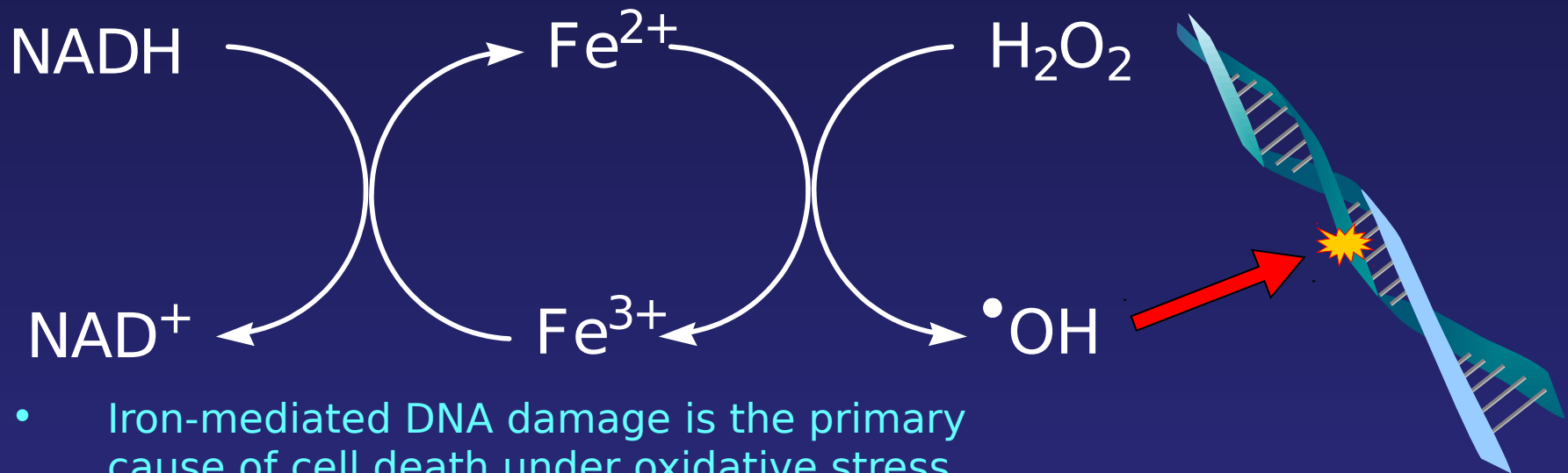


Driving the Future of Nutraceuticals

Effect of Quercetin on Rhinovirus-Induced IL-8 Elaboration in NHBE Cells



The Fenton Reaction



- Iron-mediated DNA damage is the primary cause of cell death under oxidative stress
- Inhibiting this damage may prevent tissue damage from heart attack or stroke, neurodegenerative diseases (Alzheimer's, Parkinson's), cancer, aging, fatigue and impaired recovery from exercise

Henle, E. S.; et al. *J. Biol. Chem.* **1999**, 274, 962-971; Orrenius, S.; et al. *Annu. Rev. Pharmacol. Toxicol.* **2007**, 47, 143-183; Park, S.; Imlay, J. *J. Bacteriol.* **2003**, 185, 1942-1950. Valko, M.; et al. *Chem.-Biol. Interact.* **2006**, 160, 1-40.



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